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Determination of bacterioplankton biomass, net production and growth efficiency in the Southern Ocean

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ABSTRACT: Several conversion factors are required for the estimation of bacterial biomass, net production and carbon demand from epifluorescence microscopy and measurements of [^{3} H]-thymidine and [^{3} H]-leucine incorporation rates. These conversion factors were evaluated simultaneously in mixed cultures of bacterial assemblages from the Weddell/Scotia Confluence of the Southern Ocean. The cultures were grown in the dark at +1 °C. Conversion factors were calculated for each culture by regression analyses of cumulative parameters. Average conversion factors were: 1.1×10^{18} cells per mol thymidine incorporated into cold TCA precipitate, 7.5×10^{15} μm^3 of biovolume per mol leucine incorporated into cold TCA precipitate, 0.4 pgC biomass per μm^3 of biovolume, and 40% carbon growth yield.

INTRODUCTION

Planktonic bacteria constitute an important component of pelagic ecosystems. They often account for a significant part of pelagic biomass (Ferguson & Rublee 1976, Watson et al. 1977, Williams 1981b), particularly in the open ocean (Cho & Azam 1988). They mediate a significant flux from dissolved into particulate organic matter, utilizable for eukaryote heterotrophs, and at the same time they remineralize nutrients for regenerated primary production (Sherr & Sherr 1988).

Bacterioplankton biomass and activity have been quantified by epifluorescence microscopy (Hobbie et al. 1977) and measurements of radiotracer incorporation, primarily of [^{3} H]-labelled thymidine (Fuhrman & Azam 1980), and recently also of [^{3} H]-leucine (Kirchman et al. 1986). These methodologies are specific, sensitive and easy to handle even at sea, and have been widely applied to all types of pelagic environments (Cole et al. 1988, Riemann & Bell 1990). Accuracy still remains a problem, however, since the estimating of biomass and activity in carbon units relies on several controversial conversion steps, e.g. from incorporated thymidine into cell production, from (produced) biovolume into biomass and from net into gross production (Bjørnsen & Riemann 1987, Riemann & Bell 1990).

Originally, conservative and theoretically derived conversion factors were applied (e.g. Ferguson & Rublee 1976, Watson et al. 1977, Fuhrman & Azam 1980). More recently, numerous experimental studies have produced empirical conversion factors, in order to evaluate and calibrate the methods and to meet the demand for realistic rather than conservative estimates (review in Riemann & Bell 1990). These extensive calibration studies have, however, revealed a wide range of conversion factors, and at present it seems problematic to extrapolate between geographical areas and research groups. Most calibrations have been carried out in freshwater or coastal waters, leaving methods almost unverified in the open ocean.

The methods have been calibrated in a variety of test systems, including pure cultures, mixed dilution cultures, continuous cultures, microcosms and mass balances for entire ecosystems (e.g. Fuhrman & Azam 1980, Scavia et al. 1986, Davis 1989). The different conversion steps have hitherto been evaluated separately. Possible errors may add up during the stepwise calculation of bacterial carbon biomass, production and demand.

In this study, we present a simple test system for simultaneous evaluation of all the conversion factors required for estimating bacterioplankton biomass and production (net and gross) from measurements of

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biovolume and incorporation rates of tritiated thymidine and leucine. Calibrations were done on surface water samples from the Southern Ocean during the European Polarstern Study (EPOS, leg 2) in December 1988.

MATERIALS AND METHODS

Sampling and establishment of cultures. Mixed bacterioplankton batch cultures were established from 3 stations in the Southern Ocean, representing the Weddell Sea (W), the Scotia Sea (S) and the Scotia/Weddell Confluence (C), which is a permanent frontal zone of elevated biological activity. Details of sampling conditions are given in Table 1. Water samples of about 12 l were taken by Niskin bottles and transferred to a polyethylene jar. Two batch cultures were grown from each station, one with reduced content of dissolved inorganic carbon (Batch A, 7 l), the other with unmanipulated DIC (Batch B, 2 l). Both cultures were inoculated with water filtered through 0.8 µm Nuclepore polycarbonate filters, in order to remove predators. The inoculum was diluted 10 times by particle-free seawater, obtained by filtration through 0.1 µm Nuclepore cartridge filters. For Batch A, dissolved inorganic carbon was stripped off from the particle-free water before addition to the inoculum, by acidification to pH 4, bubbling with CO_2-free air for about 3 h, and readjustment of pH to 8.0 with a buffer containing 100 mM NaOH and 10 mM $Na_2 PO_4$. Less than 2 ml of buffer was added per liter of medium, so the PO_4 concentration was increased less than 10-fold above the ambient level in the Southern Ocean of about 2µM. Batch A was grown in a closed 10 l glass bottle, and samples were taken through a tubing; air let in to replace the sample passed a 20 cm column of soda lime CO_2 absorber. Batch B was grown in a 2 l polycarbonate bottle. The batches were incubated in the dark at +1 °C, and were sampled daily for 13 d (Scotia experiment only 8 d) for determination of thymidine incorporation and cell number. Batch A was further sampled daily for determination of leucine incorporation and every third day for measurement of cell volume, particulate organic carbon and dissolved inorganic carbon.

[^{3}H]-thymidine incorporation into cold TCA precipitate. Triplicate water samples of 16 ml were incubated with 10 nM of methyl-[^{3}H]-thymidine (Amersham, 50 Ci mmol^{-1}) for 60 min. Incubations were stopped by 600 µl 39 % neutral formalin. A prekilled sample was used as blank. The samples were filtered onto 0.22 µm cellulose acetate filters, rinsed 10 times with 1 ml 5 % ice-cold trichloroacetic acid (TCA) and radioassayed on board in an LKB 1209 Rack-Beta liquid scintillation counter, using PCS (Amersham) as scintillator. The dependence of thymidine incorporation on incubation time and [^{3}H]-thymidine concentration was assayed on the original, unmanipulated water samples immediately after sampling. For the Scotia Sea experiment, however, this assay was done 17 d before the batch experiment, but from the same geographical position.

[^{3}H]-leucine incorporation into cold TCA precipitate. Triplicate water samples of 16 ml were incubated for 60 min with 8.5 nM of L-[3,4,5-^{3}H(N)]-leucine (NEN, specific activity diluted to 73.6 Ci or 16.36 Ci mmol^{-1}). Otherwise, the procedure parallelled that described above for measuring thymidine incorporation.

Cell counts and cell volume. Bacterioplankton cells were counted and sized under epifluorescence microscope (Hobbie et al. 1977). Water samples of 16 ml were preserved with 0.6 ml of particle-free neutral formalin (39 %). Subsamples of 2 ml were mixed with 4 ml of 0.2 µm filtered demineralised water and filtered onto black 0.2 µm Nuclepore filters (25 mm diam). The filters were covered with Acridine Orange solution (1 g l^{-1}). After 5 min of staining, the filters were sucked dry, and stored in the dark. Bacteria were counted and sized under a Leitz Dialux Laborlux D epifluorescence microscope under blue light (filter set I2/3). Cell length and width were measured by comparison to the globes of a calibrated New Porton grid (Graticules Ltd). Cell volumes were calculated according to the formula given in Fuhrman (1981). The measures were always taken from the inner bright edge of the cells, so the volume estimates presented here are likely to be conservative.

Table 1. Sampling conditions for batch experiments ('B') and evaluations of isotope concentration and incubation time ('CT'). Ambient temperatures (Temp.) and chlorophyll *a* (Chl *a*) concentrations from C. Veth & G. Jaques (pers. comm.)

Experiment	Date of sampling	Position	Temp. (°C)	Chl *a* (µg l^{-1})	Station
Weddell Sea (B & CT)	22 Dec 1988	61°00 S, 49°00 W	−1.6	0.8	14/176
Confluence (B & CT)	08 Dec 1988	59°25 S, 48°45 W	−1.3	2.0	14/158
Scotia Sea (B)	27 Dec 1988	57°00 S, 49°00 W	2.6	0.6	14/182
(CT)	10 Dec 1988	– –	1.2	0.3	14/159

Particulate organic carbon (POC). Triplicate 50 ml water samples were filtered onto three 6 mm diameter spots on a precombusted (at 500 °C) 47 mm GF/F disc (Whatman). Two triplicate filtrations were carried out for each sample. The filters were dried at 40 °C and stored for combustion infrared gas analysis. The spots containing the samples were punched out with a 9 mm diameter hollow cork drill. A 9 mm disc was taken from the center of the filter and used as a blank. Each disc was combusted at 960 °C for 8 s in a flow of oxygen that carried the produced CO_2 to a Hartman-Braun infrared gas analyser equipped with a Hewlett-Packard integrator. Pre-weighed crystals of oxalic acid were used as a standard.

Dissolved inorganic carbon (DIC). Five replicate subsamples of 150 µl were injected into a chamber containing 3 ml of 3 % HNO_3, bubbled by a nitrogen flow (500 ml min^{-1}), that carried the released CO_2 to an infrared gas analyser (ADC, model 225 MK 3). The signal was integrated over a period of 75 s, and measurements were calibrated against solutions of $KHCO_3$. The infrared gas analyser, which is sensitive to displacements and vibrations, was placed close to the ship's center of gravity, and analysis was carried out only when the ship was at station.

Data handling. Conversion factors were derived by linear regression of cumulative parameters. Thus, a conversion factor from thymidine incorporation rate to cell production rate was estimated for each batch as the slope of the regression between cumulative incorporated thymidine and cell number. Similarly, conversion factors from leucine incorporation to biovolume production, from biovolume to carbon biomass and from gross to net carbon production (growth yield) were estimated from regression analyses between cumulative leucine incorporation, biovolume, POC (cumulative net carbon production) and POC+DIC (cumulative gross carbon production).

RESULTS AND DISCUSSION

Thymidine incorporation

Thymidine incorporation was proportional to incubation time for at least 2 h, and was almost constant for

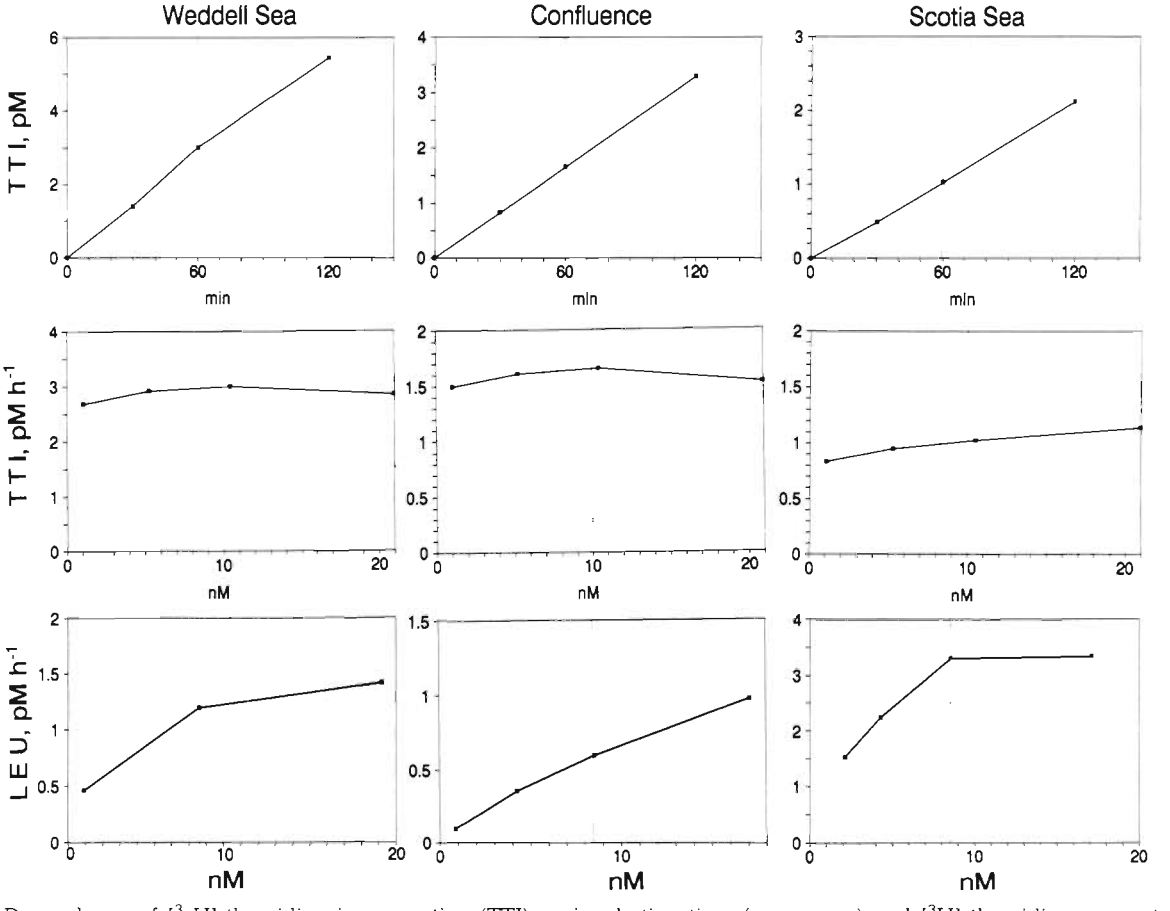

Fig. 1. Dependence of [^{3} H]-thymidine incorporation (TTI) on incubation time (upper row) and [^{3}H]-thymidine concentration (middle row); dependence of [^{3}H]-leucine incorporation (LEU) on [^{3}H]-leucine concentration (lower row). Vertical dotted lines indicate conditions used in experiments

[^{3}H]-thymidine concentrations between 1 and 20 nM (Fig. 1). Thus, our use of 1 h incubations and 10 nM [^{3}H]-thymidine represented a choice within wide safety margins. Since blank values are roughly proportional to the [^{3}H]-thymidine concentration (data not shown), the sample-to-blank ratio and thereby the sensitivity of the method could have been improved by an order of magnitude by using 2 h incubation and 2 nM [^{3}H]-thymidine addition. The sensitivity of our procedure was about 0.1 pM h^{-1}.

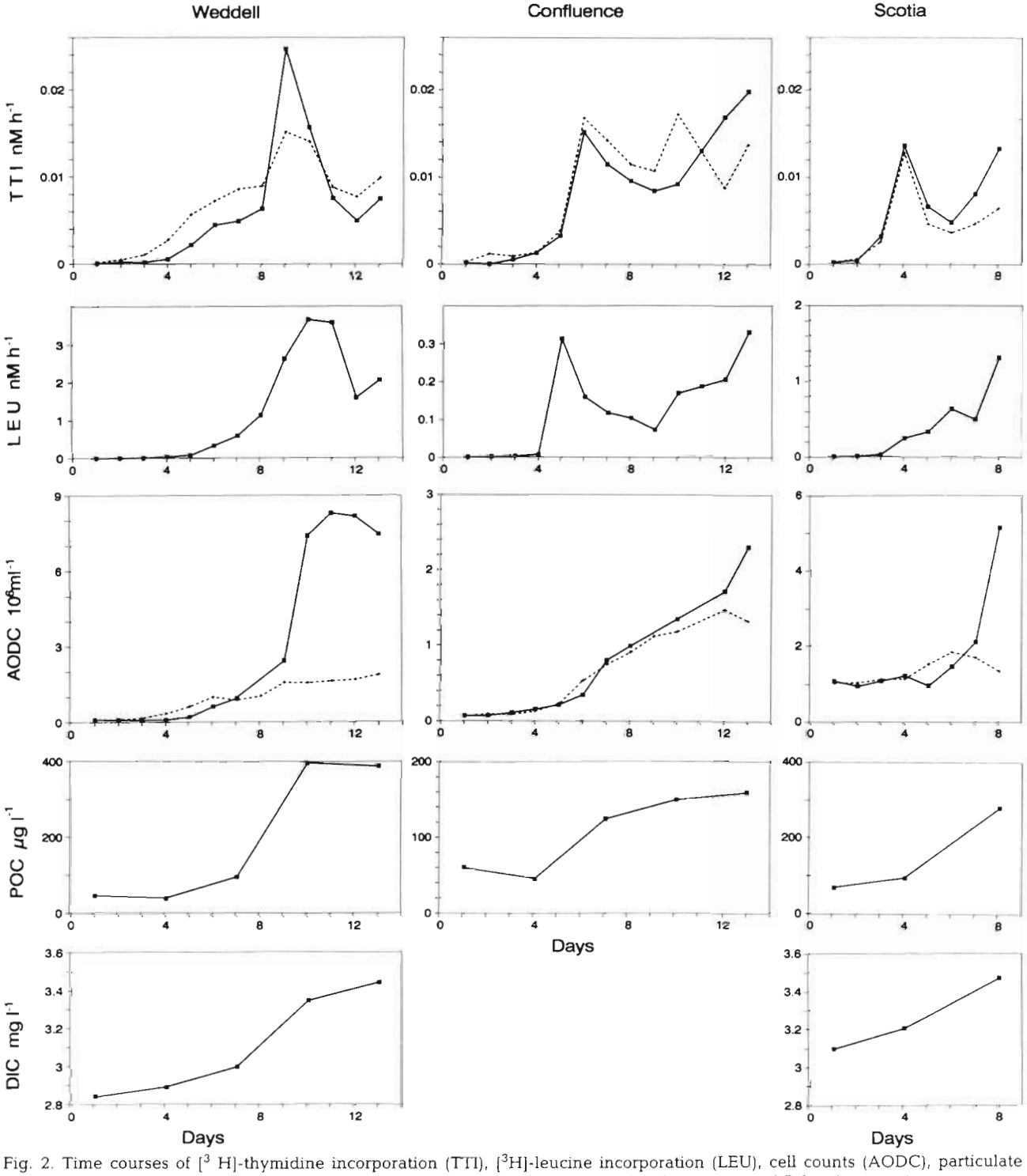

Fig. 2. Time courses of [^{3} H]-thymidine incorporation (TTI), [^{3}H]-leucine incorporation (LEU), cell counts (AODC), particulate organic carbon (POC), and dissolved organic carbon (DIC) in A-batches (——) and B-batches (- - - -)

Thymidine and leucine incorporation rates increased exponentially in all batch cultures for 4 to 8 d with increase rates of 0.5 to 1.8 d^{-1}, and with no apparent time lag at the beginning of the experiments (Figs. 2 & 3). Leucine incorporation rate increased more rapidly than thymidine incorporation rate, but the difference was significant only in the Weddell experiment. Cell numbers increased at much slower rates (0.0 to 0.4 d^{-1}, data not shown), suggesting that not all cells were active. Cell volumes increased significantly during the first 3 d of the experiments, then stabilised or decreased (Table 2). The proportion of rod-shaped bacteria (as opposed to cocci) increased from about 60 % at the start to about 90 % at the end of the experiments (data not shown). These observations also indicate some selectivity and/or enrichment of the batches. Increase of cell volume is often observed in response to confinement of bacterioplankton (Ferguson et al. 1984) and may be due to enrichment and lack of predation (Gonzales et al. 1990).

Thymidine incorporation rate were almost similar in the 2 types of batches (A vs B) throughout the experiments (Fig. 2). Cell numbers developed similarly in A- and B-batches for the first 7 to 10 d, but then A-batches with reduced DIC showed rapid increases of cell numbers, particularly in the Weddell and Scotia experiments. The carrying capacity in the B-batches of about 5 to 10 µmol organic carbon l^{-1} was probably not limited by available phosphate (ca 2 µM), so it seems unlikely that this stimulation of the A-batches was a direct effect of the phosphate addition (<20 µM). The acidification may, however, have hydrolyzed high molecular weight dissolved organic compounds, which thereby became more readily available for the bacteria.

Thymidine incorporation was integrated from Day 1 (Table 2) and compared by linear regression to cell numbers (Fig. 4), in order to reveal an empirical conversion factor from [^{3}H]-thymide incorporation (TTI) to cell production for each individual batch (Table 3). Some data points were excluded in these regression analyses (Fig. 4), either because cell numbers started to

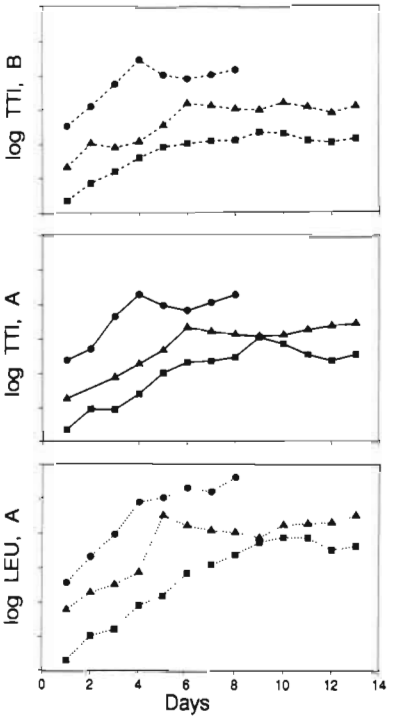

Fig. 3. Logarithmic time courses of TTI in Batches A (——) and B (----), and of LEU in Batch A (·····). The curves have been placed arbitrarily in relation to the ordinate to facilitate visual separation

Table 2. Integrated [^{3}H]-thymidine and [^{3}H]-leucine incorporation (ΣTTI and ΣLEU), cell counts and volumes (AODC and Vol), and particulate organic carbon and dissolved inorganic carbon (POC and DIC) from the batch experiments (Batch A with reduced DIC content, Batch B unmanipulated)

Experiment	Day	Batch A						Batch B	
		ΣTTI (nM)	ΣLEU (nM)	AODC (10^9 l^{-1})	Vol (µm^3)	POC (µg l^{-1})	DIC (µg l^{-1})	ΣTTI (nM)	AODC (10^9 l^{-1})
Weddell	1	–	–	0.10	0.076	46	2845	–	0.11
	4	0.02	0.8	0.10	0.100	39	2890	0.07	0.35
	7	0.24	18.0	0.94	0.122	96	3000	0.52	0.89
	10	1.23	159.3	7.40	0.107	397	3350	1.37	1.56
	13	1.82	352.4	7.46	0.092	390	3445	2.06	1.88
Confluence	1	–	–	0.07	0.079	61	ND	–	0.06
	4	0.03	0.2	0.16	0.153	45	ND	0.07	0.13
	7	0.62	13.0	0.81	0.146	124	ND	0.75	0.75
	10	1.30	20.6	1.35	0.136	150	ND	1.66	1.19
	13	2.36	36.0	2.30	0.136	158	ND	2.55	1.32
Scotia	1	–	–	1.06	0.114	68	3095	–	1.01
	4	0.25	3.7	1.21	0.165	93	3205	0.23	1.13
	8	1.03	57.1	5.12	0.143	279	3470	0.76	1.32

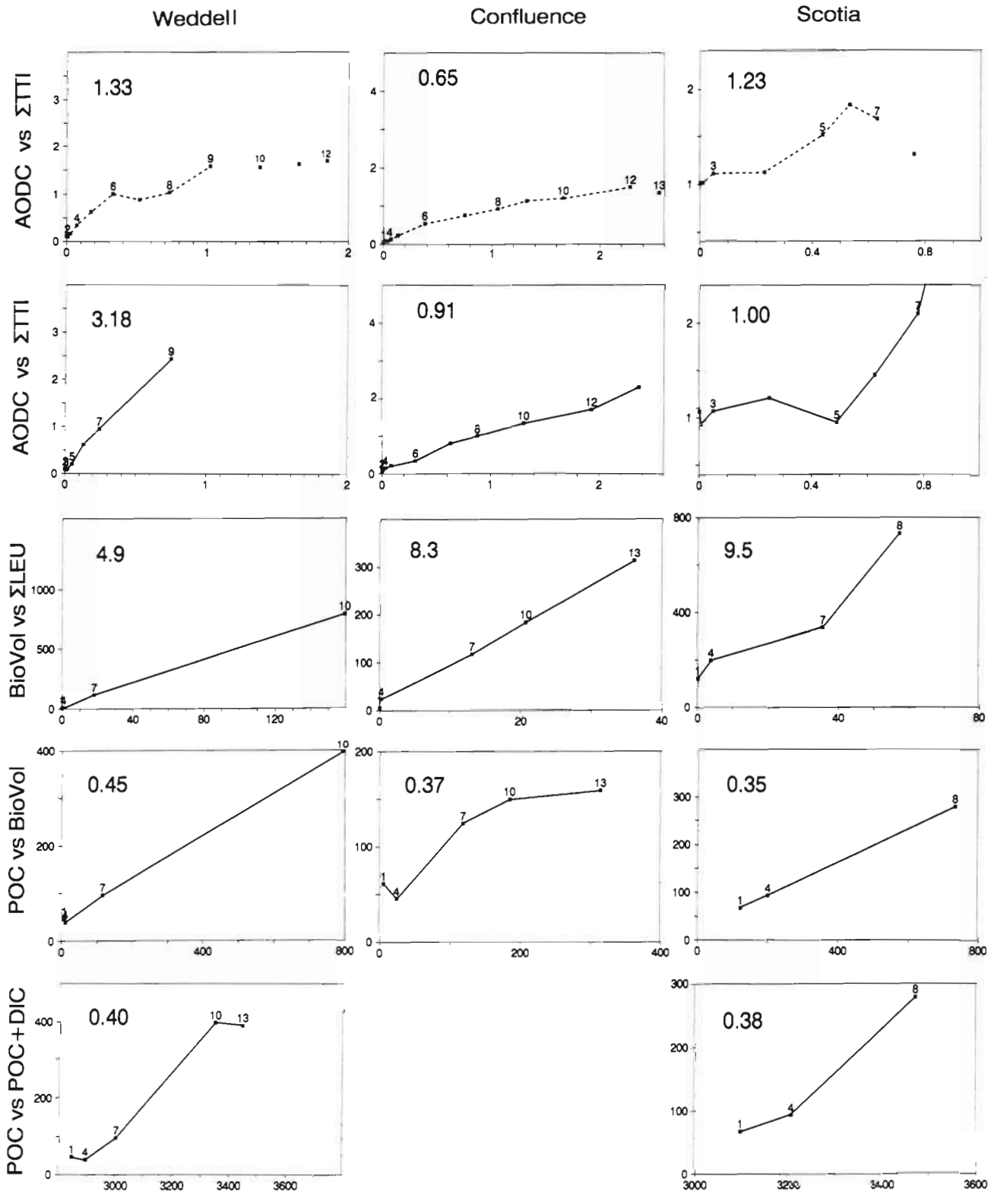

Fig. 4. Regression of cumulative parameters (A-batches: ——, B-batches: ----). Point labels refer to sampling days. Numbers in upper left of each box give slopes of linear regressions representing conversion factors from TTI to cell production (10^{18} cells mol^{-1}), from LEU to biovolume production (10^{15} μm^3 mol^{-1}), from biovolume to carbon biomass (pg C μm^{-3}) and from gross to net carbon production (carbon growth yield)

decrease, or because cell numbers showed rapid increases that were apparently not reflected in the TTI measurements (Fig. 2). The regression analyses produced conversion factors that differed significantly from each other (Table 3). In particular, 2 batches, W-A and C-B (3.18 and 0.65 $\times 10^{18}$ cells mol^{-1}, respectively), deviated remarkably from the remaining 4 batches, where the conversion factors clustered around a mean value of 1.12 $\times 10^{18}$ cells mol^{-1}. The overall average for all batches was 1.37 $\times 10^{18}$ cells mol^{-1}. These values are consistent with empirical conversion factors found in subtropical and temperate marine waters. Thus Fuhrman & Azam (1982) and Riemann et al. (1987) found conversion factors of 1.1 to 1.4 $\times 10^{18}$ cells mol^{-1} [^{3}H]-thymidine incorporation into macromolecular material. In the latter study, 3 out of 65 values were, however, significantly higher: about 3.8 to 5.8 $\times 10^{18}$ cells mol^{-1}. Kirchman & Hoch (1988) arrived at a conversion factor of 1.0 $\times 10^{18}$ cells mol^{-1}. Higher conversion factors have been obtained in freshwater (e.g. Bell et al. 1983, Lovell & Konopka 1985, Smits & Riemann 1988, Riemann & Bell 1990), particularly at high growth rates (Smits & Riemann 1988). Coveney & Wetzel (1988) found that conversion of thymidine incorporation into bacterial biovolume production was less variable than conversion into cell production in oligotrophic lake water cultures. In this study, conversion factors of 6.44, 1.26 and 5.25 $\times 10^{17}$ μm^3 mol^{-1} were derived from the Weddell, Confluence and Scotia experiments, respectively. The first and last values are very close to the average of 5.54 $\times 10^{17}$ μm^3 mol^{-1} found by Coveney & Wetzel (1988), while the result from the Confluence experiment was significantly lower.

Different calculation protocols have been applied to extract empirical conversion factor from simultaneous measurements of cell number increase and thymidine incorporation in dilution cultures of bacterioplankton. Fuhrman & Azam (1980, 1982) directly compared cell number increase over 24 h to the initial thymidine incorporation rate, assuming constant incorporation rate throughout the experiment. Kirchman et al. (1982) introduced a derivative calculation method based on regression analysis of logarithmic plots against time, assuming that all cells grow exponentially and at a similar rate. Riemann et al. (1987) calculated the conversion factor as cell number increase divided by integrated thymidine incorporation during a given period. This integrative method is model-free, but does not fully exploit the data set; only start and end cell numbers are utilized and therefore need to be very precisely determined.

In this paper, we have used what could be called a *cumulative* method, based on a direct regression between integrated values, in this case cell number and integrated thymidine incorporation. This method combines the advantages of the derivative and integrative methods: it extracts maximum information from the data set (all data points are considered in the regression analysis), with a minimum of presumptions (no growth model is required since the time parameter is cancelled out). The constancy of the conversion factor during the experiment can easily be verified from the linearity of the regression plot (Fig. 4), as expressed by the regression coefficient (Table 3).

Leucine incorporation

The determination of bacterioplankton production from measurements of [^{3}H]-leucine incorporation into

Table 3. Conversion factors derived by linear regression of cumulative parameters, cf. Fig. 4. Conversion factors (CF), standard errors (±SE), number of data points (n) and regression coefficients (r^2) are given for each experiment. W: Weddell; C: Confluence; S: Scotia

Conversion factor (unit)	Expt	CF	(±SE)	n	r^2
TTI to cell production	W-A	3.18	(± 0.10)	8	0.99
(10^{18} cells mol^{-1})	W-B	1.33	(± 0.17)	9	0.90
	C-A	0.91	(± 0.03)	11	0.99
	C-B	0.65	(± 0.04)	11	0.96
	S-A	1.00	(± 0.37)	7	0.60
	S-B	1.23	(± 0.18)	7	0.90
LEU Biovol. prod.	W-A	4.90	(± 0.09)	4	0.99
(10^{15} μm^3 mol^{-1})	C-A	8.31	(± 0.24)	5	0.99
	S-A	9.51	(± 0.21)	3	0.90
Biovol. to C biomass	W-A	0.45	(± 0.01)	4	0.99
(pg C μm^{-3})	C-A	0.37	(± 0.10)	5	0.84
	S-A	0.35	(± 0.01)	3	0.99
Carbon growth yield	W-A	0.40	(± 0.02)	5	0.99
(C/C)	S-A	0.38	(± 0.04)	3	0.99

bacterial protein has been introduced as an alternative to the now widely used [^{3}H]-thymidine incorporation (TTI) method. Theoretically, thymidine is mainly incorporated into DNA, which is closely related to cell number, while leucine is mainly incorporated into protein, which is related to biomass. Thus, the leucine method seems to offer an attractive shortcut to an estimate of bacterial carbon production, compared to the stepwise conversion of thymidine incorporation via cell production and biovolume production.

On the other hand, several methodological aspects of the leucine method still appear problematic. Often, a high external concentration of [^{3}H]-leucine is required to saturate incorporation (Fig. 1; Kirchman et al. 1986, Kirchman & Hoch 1988). This indicates that de novo synthesis of leucine takes place to a considerable extent, as also shown directly by Simon and Azam (1988), and the use of high [^{3}H]-leucine concentrations increases the risk that organisms other than bacteria may incorporate the tracer. The constancy of leucine mol percentage in bacterial protein [Hagström et al. 1984 (8.3 to 9.1 %), Simon & Azam 1988 (7.3 to 1.9 %, n = 47)] also needs verification from a wider range of aquatic environments. In this study, extraction in cold TCA instead of boiling TCA (Simon & Azam 1988) was chosen for practical reasons. Chin-Leo & Kirchman (1988) found no difference between the 2 procedures.

The available information on empirical conversion factors for leucine incorporation to bacterial production is much more limited than for thymidine incorporation, and only a few studies convert into biovolume or biomass rather than to cell production. Simon & Azam (1988) found a conversion factor to biomass production of 3.1 kgC mol^{-1} (assuming isotope dilution of 2 and C/protein = 0.86 wt/wt), which is almost identical to the average of our conversion factors (7.5×10^{15} µm^3 mol^{-1} × 0.4 pgC µm^{-3} = 3.0 kgC mol^{-1}). Kirchman et al. (1986) obtained conversion factors of 1.1 to 9.5 kgC mol^{-1} from leucine incorporation rates at [^{3}H]-leucine concentrations of 10 nM or at saturation.

Cell carbon

The regression analyses of POC versus biovolume revealed an average conversion factor of 0.4 pgC µm^{-3}. The traditionally used carbon densities of 0.086 to 0.121 pgC µm^{-3} (Ferguson & Rublee 1976, Watson et al. 1977) have been criticised for their general lack of empirical foundation (Bjørnsen & Riemann 1987), and specifically for not addressing the problem of intercellular water when determining dry-to-wet-weight ratios (Bratbak & Dundas 1984). Several recent studies have related biovolumes to directly measured carbon biomasses, revealing a wide range of empirical conversion factors [e.g. 0.56 pgC µm^{-3} (Bratbak 1985), 0.11 pgC µm^{-3} (Nagata 1986), 0.35 pgC µm^{-3} (Bjørnsen 1986a) and 0.38 pgC µm^{-3} (Lee & Fuhrman 1987)]. A calibration study in several pelagic environments using the same sizing procedure as in this study produced an average carbon density of 0.38 pgC µm^{-3} (Salonen et al. unpubl.). The variability in empirical carbon-to-volume ratios is probably caused primarily by the inherent subjectivity in microscopic sizing of bacterial cells. The comparability of the empirically found carbon densities could be improved by an intercalibration among the laboratories involved.

Growth yield

Carbon growth yields of 0.40 and 0.38 were estimated for the Weddell and Scotia experiments. Unfortunately, the infrared gas analyser was not in operation during the Confluence experiment. The literature on bacterioplankton growth yield is characterized by controversy. The high uptake efficiencies (50 to 90 %) found after short incubations of bacterioplankton with radiolabelled substrates (Williams 1984) reflect assimilation efficiencies rather than growth efficiencies (Bjørnsen & Riemann 1987). Growth efficiencies of 10 to 30 % have been estimated from comparisons of bacterial net production with either respiration or substrate consumption (refs. in Bjørnsen 1986b). Most of these estimates, however, are indirect and rely on other conversion factors, and particularly net production may have been underestimated by using conservative carbon densities. A comparison of POC and DIC production in continuous cultures of bacteria from a Danish estuary (Bjørnsen 1986b) revealed a growth yield of 21 %, while a regression analysis of pelagic community respiration in the same estuary suggested a bacterial growth yield of 42 % (Jensen et al. 1990). Bacterioplankton growth yield is a crucial parameter for the establishment of pelagic carbon budgets. If a low bacterial growth yield (e.g. <25 %) is assumed, it becomes difficult to accomplish bacterioplankton carbon demand by autochtonous sources (review by Cole et al. 1988). On the other hand, a high bacterial growth yield (>50 %) is inconsistent with the observations of bacterioplankton being responsible for more than half of the pelagic community respiration (e.g. Williams 1981a, Schwaerter et al. 1988, Jensen et al. 1990).

The test system

The test system described in this paper allowed simultaneous evaluation of all the conversion factors required to convert thymidine (TTI) and leucine (LEU)

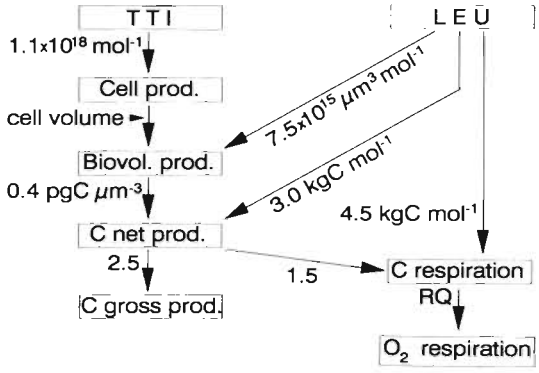

Fig. 5. Summary of average empirical conversion factors found in this study from [^{3}H]-thymidine incorporation (TTI) and [^{3}H]-leucine incorporation (LEU) into bacterial production, carbon demand and respiration

incorporation into bacterial net production, carbon demand and respiration (Fig. 5). The obtained empirical conversion factors are consistent with experiences from temperate and subtropical coastal waters. For the TTI method we suggest a conservative conversion factor of 1.1×10^{18} cells mol^{-1}, noting that this value may occasionally underestimate bacterial net production. The carbon density of 0.4 pgC µm^{-3} probably depends on the cell sizing procedure and should only be applied to other procedures after proper intercalibration.

The conversion of leucine incorporation to biovolume or biomass production (3.0 kgC mol^{-1}) represents an attractive shortcut in avoiding cell sizing, but experiences with this method are still limited. High saturation concentrations of leucine represent a potential methodological problem. At present the leucine incorporation method therefore appears more as a supplement than as an alternative to the TTI method.

The conversion of net production into gross production and respiration by assuming a carbon growth yield of 40 % appears as a realistic compromise among the conflicting literature values of 10 to 70 %. The conversion of carbon respiration into oxygen consumption is relevant for comparisons to measured oxygen consumption rates and for establishing oxygen budgets, but unfortunately little is known about respiratoric quotients of the utilizable dissolved organic compounds in pelagic environments.

Any test system based on fractionation and confinement may selectively alter the growth conditions (Ferguson et al. 1984, Coveney & Wetzel 1988), and therefore manipulations and incubation time should be kept at a minimum. In this study we tried to design the test system to be as simple as possible. The relatively long duration of the experiments was required at the low temperatures, and the CO_2 removal in the A-batches was necessary to achieve sufficient precision of the DIC measurements. The bacteria responded to the manipulations by active growth, which made it possible to extract empirical conversion factors. Similar thymidine conversion factors were derived from A- and B-batches, despite the observed differences in bacterial growth.

There is need for further calibration of the thymidine, leucine and fluorochrome methods, particularly in oceanic environments, before bacterioplankton production and biomass can be assessed on a routine basis. The consistency of our results with the literature on calibrations in coastal waters does, however, strongly support the usefulness and reliability of the tested methods, even in the – from any point of view – extreme environment of the Southern Ocean.

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